

# BEST AVAILABLE COPY

Expand 4 cultures from Jones from 1/2 ml to 10 ml 37°C shaker  
to make glycerol stocks of all 4 grow 1 clone at CN# 2 300 ml

CN88 clone 1 18.68.1	200 ml culture	LB
CN88 clone 2 18.68.3	10 ml Min	
CN89 clone 1 18.68.2	200 ml culture	
CN89 clone 2 18.68.4	10 ml Min	

CN 88 = 6kb PSE from CDI in BS+ as Hind III Xba I @ 5' end of PSE

CN 89 = 6kb PSE from CDI in BS+ as Hind III Sac I @ 5' end of PSE

Qigen Min CN88 clone 1 & CN89 clone 1 16p 20, 2 min, QBT 1x30 ml, QC 2x24-48  
10 ml Min prep alk lysis CN88 clone 2 CN89 clone 2

Tube	DNA	Wet	10x NEB Buffer	10x BSA	Alt. C	Enzyme
1	CN88 clone 1		2	1 ul		-
2	"					Hind III
3	"					Xba I
34	CN88 clone 2					-
5	"					Hind III
6	"					Xba I
7	CN89 clone 1					-
8	"					Hind III
9	"					Xba I
10	CN89 clone 2					-
11	"					Hind III
12	"					Xba I

see to cloning this CN88 clone 1 is good

Transform into DH5+ cells to grow DNA for stock

BHG-1064 16.192  
BHG-1164 16.192  
CN84.1 18.63

Make SB Media for BHG cultures

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Witnessed & Understood by me,

H. Lam p. 1/2

Date

11/1/95

Invented by

Recorded by

H. Lam

Date

7/8/95

## BEST AVAILABLE COPY

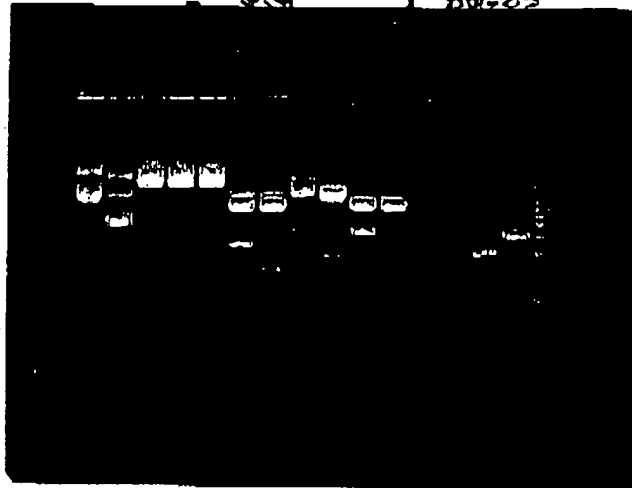
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Restriction digest

Tube	DNA	6cl	10X NBS Buffer	10X PSA	clH2O	Enzyme
1	CN78	7ul ✓	1 ✓	0	2, 1 ✓	+
2			1 ✓		1 ✓	NarI ✓
3			2			HindIII ✓ linear
4			EcoRI ✓			EcoRI ✓ linear
5			✓			BglII ✓ linear
6			✓		0	EcoRI/BglII ✓ 3200, 6409
7			✓		0	EcoRI/BglII/HindIII ✓ 900, 2300, 6409
8	CN85	2ul ✓	2 ✓	1ul ✓	5 ✓	+
9					5 ✓	ClaI ✓ 2,760, 7500
10						XbaI ✓
11						ClaI/XbaI ✓ 158, 2725, 6409
12	BHG-83-1	4ul ✓	2 ✓	0	4 ✓	+
13	"				4 ✓	HindIII ✓
14	BHG-83-2	4ul ✓			7 ✓	+
15	"				7 ✓	HindIII ✓ to 315

CN78 looks fine  
 CN85 looks fine  
 BHG-83's must have forgotten enzyme change

1 BHG-83



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Witnessed &amp; Understood by me,

H. Lamparky

Date

5/16/95

Invented by

5/16/95

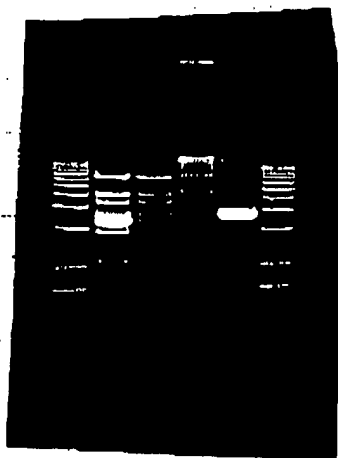
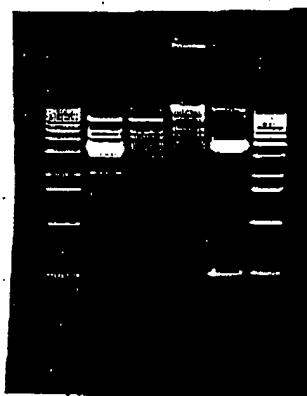
Date

5/16/95

## BEST AVAILABLE COPY

Tube	DNase	vol	Ex	PCR	CHC	Enzyme
A	BHG 10 20.4	1ul	2	0	7ul	Hind III ✓
B	BHG 11 20.4	1ul	2	0	7ul	Hind III ✓
C	BHG E3 1 20.1	8ul	2	0	7ul	Hind III ✓
D	BHG E3 2 20.1	1ul	2	0	7ul	Hind III ✓

1	CP 76	2ul	2	1ul	5ul	Superficial
2	16.192	2ul	2	1ul	5ul	Hind III ✓
3	11.20	1ul	1	1ul	5ul	SacI 16.1, 5.8
4		2ul	2	1ul	5ul	XbaI 3880, 2986
5		2ul	2	1ul	5ul	ClaI 1156, 5244 To 10-11



BHG 10 still looks good the large band  
 at 3+ Kb is too large  
 BHG 11 looks good  
 BHG E3-1 has the correct bands but is  
 smearing!  
 BHG E3-2 is trash



CN 76 looks fine!  
 Accel OD & Gel in 1.5 ml purple rack -20°C chest

CN 76 16.192 OD 26.0 2580 264/280 Conc  
 0.402 0.219 1.8 4 mg/ml

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Witnessed &amp; Understood by me,

M. Lamparky

Date

5/10/95

Invented by

Recorded by

Date

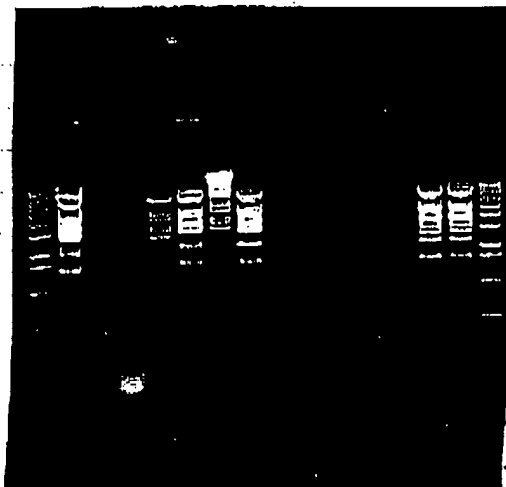
5/10/95

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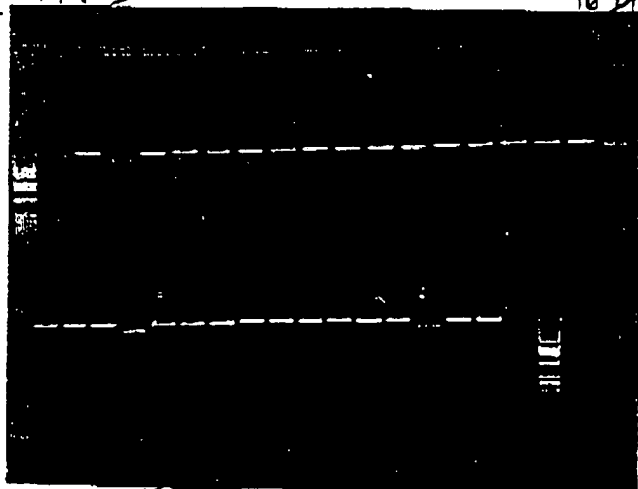
Restriction Digest BHC's

File	DNA	NGS #22	dt20	Language
1	BHG10-1 20.07	0.540	1	7.5
2	BHG10-2 20.07	6	2	1.1HindIII 2.464
3	BHG10-3 20.07	6	2	
4	BHG10-4 20.08	4	2	
5	BHG10-1 20.10	4	4	
6	BHG10-2 20.10	4	4	
7	BHG10-3 20.10	4	4	
8	BHG10-1 20.08	6	2	
9	BHG10-2 20.08	6	2	
10	BHG10-3 20.08	6	2	
11	BHG10-1 20.08	6	2	
12	BHG10-2 20.10	4	4	
13	BHG10-3 20.10	4	4	
14	BHG10-1 20.10	4	4	



1-33 15.106.016 #1 Sub Age1 7.0 1150

(parent) 1 2



BHG10-20.07-12 BHG10-20.10  
seem to run differently pool  
separately & redigest - dilute 20.07's  
pool 11's & redigest

These clones look great nearly  
all cut with Age1. have insert  
#2, 17, 21 & 31 are insert - trash

I will further characterize 1 & 16  
with  
XbaI  
EcoRI  
XbaI/EcoRI  
Age1  
XbaI/EcoRI/Age1  
EcoRI/Age1  
XbaI/Age1

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Witnessed &amp; Understood by me,

Date

5/30/95

Invented by

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Date

4/17/95